Table S1. Clinical Isolates used in this study

Isolate ID	Organism source	Date of Isolation
KP1	Blood Culture	12/04/2016
KP2	Blood Culture	08/03/2016
KP3	Tissue	10/01/2016
KP4	Tissue	07/12/2015
KP5	Blood Culture	05/10/2015
KP6	Blood Culture	25/09/2015
KP7	Blood Culture	01/09/2015
KP8	Ascitic Fluid	23/02/2015
KP9	Tissue/Kidney Stone	05/12/2014
KP10	Blood Culture	27/10/2014
KP11	Screening Swab	10/10/2014
KP12	Blood Culture	01/10/2014
KP13	Groin swab	02/07/2014
KP14	Wound swab	11/06/2014
KP15	Blood Culture	20/05/2014
KP16	Blood Culture	20/04/2014
KP17	Sputum	19/03/2014
KP18	Catheter urine	18/02/2014
KP19	Catheter urine	17/10/2013
KP20	Sputum	01/08/2013
KP21	Sputum	30/07/2013
KP22	Blood Culture	27/07/2013
KP23	Wound swab	23/07/2013
KP24	Mid-stream urine	23/07/2013
KP25	Blood Culture	22/07/2013
KP26	Mid-stream urine	03/07/2013
KP27	Central Line Tip	17/04/2013
KP28	Bronchio-alveolar Lavage (BAL)	11/03/2013
KP30	Blood Culture	07/08/2012
KP31	Blood Culture	06/07/2016
KP32	Tissue	05/07/2016
KP33	Pus	05/07/2016
KP34	Tissue	28/06/2016
KP38	Blood Culture	21/06/2016
KP40	Blood Culture	09/06/2016

KP46	Tissue	22/04/2016
KP47	Blood Culture	22/04/2016
KP48	Peritoneal Fluid	01/04/2016
KP50	Pus	23/03/2016
KP59	Blood Culture	25/02/2016

Table S2. PCR Primers used in this study

Primer	Sequence (5' to 3')	Reference
gyrA - F	AATATGCGCCATCAGCCC	(S1)
gyrA - R	TGCGAGAGAAATTACACC	(S1)
parC - F	ATGAGCGATATGGCAGAGC	This work
parC - R	CCACTTCCCGCAGGTTGT	This work
oqxR - F	TTCCTGACGCCGGTGTTTTA	(S2)
oqxR - R	CTGCGGTGCCAAAAAGAACA	(S2)
ramR - F	CTGCAGTGCCCGGTGAACCCTGGCGT	(S3)
ramR - R	CTGCAGATTTGCTGATTCAGCAGCGAC	(S3)
Qnr synthesis	From: GAGCGCTTGTGTCGCTACAT To: GGTTGCTTCATGGGCAA	As Accession number AY070235.1
AAC-6'-Ib-cr synthesis	From: GAGCAAACGATCAATGCG To: AAGCCTAGCCAATTTGCTAGG	As Accession number JF775514.1

Table S3. Mutations identified in the *oqxR*

Mutant/Isolate	Amino acid position and changes
oqxR1	Tyr109STOP
oqxR2	Leu89FS
oqxR3	Ala37Glu
oqxR4	Ala133Val
oqxR5	Arg93Cys
oqxR6	Glu85STOP
oqxR7	Gln21STOP
oqxR8	Glu116STOP
oqxR9	Asp3Glu
oqxR10	Ala133Pro
oqxR11	Ala37Glu
oqxR12	Gly63 ins SIHLGRNG
KP9	Glu24STOP
KP12	Asp3Tyr
KP20	Ala19Val
KP28	Leu76FS
KP30	Val130Ala

Effects on OqxR sequence of mutations identified in *oqxR* in Ecl8 mutants (alleles *oqxR1* to 12) or in the *K. pneumoniae* clinical isolates (KP9, 12 etc). All these mutations have been shown to result in loss of function, and so OqxAB hyper-production.

Table S4: Disc susceptibility testing according to CLSI protocol (26) for *K. pneumoniae* Ecl8 and mutants determining the effect of acquisition events on Fluoroquinolone susceptibility.

Parental strain[a], mutantion (transformed plasmid)	Susceptiblity/Non-Suscerptibility to CIP	LEV	NOR	OFL	Mutation/Transformation
Ecl8	**S**	**S**	**S**	**S**	**Wildtype**
Ecl8 (pEX-K4)	**S**	**S**	**S**	**S**	**Vector only**
<u>Single acquisition events</u>					
gyrA1	S	S	S	S	GyrA:Ser83Phe
gyrA2	S	S	S	S	GyrA Ser83Tyr
gyrA3	S	S	S	S	GyrA Asp87Tyr
oqxR1	S	S	S	S	OqxR:Tyr109STOP
oqxR2	S	S	S	S	OqxR:Leu89FS
oqxR3	S	S	S	S	OqxR:Ala37Glu
oqxR4	S	S	S	S	OqxR:Ala133Val
ΔramR	S	S	S	S	deleted RamR
ramR	S	S	S	S	RamR:Thr124Pro
(qnrA1)	S	S	S	S	QnrA1
(aac(6')-Ib-cr)	S	S	S	S	Aac(6')-Ib-cr
<u>Double acquisition events</u>					
oqxR1 + gyrA2	NS	NS	NS	NS	OqxR:Tyr109STOP + GyrA:Ser83Tyr
ΔramR + gyrA1	NS	NS	S	NS	deleted RamR + GyrA:Ser83Phe
ΔramR + oqxR5	S	S	NS	S	deleted RamR + OqxR:Arg93Cys
(qnrA1 + aac(6')-Ib-cr)	S	S	S	S	QnrA1+ Aac(6')-Ib-cr
***gyrA1* (pEX-K4)**	S	S	S	S	GyrA:Ser83Phe Vector only
gyrA1 (qnrA1)	S	S	S	S	GyrA:Ser83Phe + QnrA1
gyrA1* (*aac(6')-Ib-cr)	S	S	S	S	GyrA:Ser83Phe + Aac(6')-Ib-cr

***oqxR1* (pEX-K4)**	S	S	S	S	OqxR:Tyr109STOP Vector only
oqxR1* *(qnrA1)	NS	NS	NS	NS	OqxR:Tyr109STOP + QnrA1
oqxR1* *(aac(6')-Ib-cr)	S	S	S	S	OqxR:Tyr109STOP + Aac(6')-Ib-cr
Δ*ramR* (pEX-K4)	S	S	S	S	deleted RamR Vector only
Δ*ramR* *(qnrA1)*	NS	NS	NS	NS	deleted RamR + QnrA1
Δ*ramR* *(aac(6')-Ib-cr)*	S	S	S	S	deleted RamR + Aac(6')-Ib-cr

Triple acquisition events

Δ*ramR* + *oqxR5* + *gyrA1*	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + GyrA:Ser83Phe
gyrA1* *(qnrA1* + *aac(6')-Ib-cr)	NS	S	NS	S	GyrA:Ser83Phe + QnrA1 + Aac(6')-Ib-cr
oqxR1* *(qnrA1* + *aac(6')-Ib-cr)	NS	S	NS	NS	OqxR:Tyr109STOP + QnrA1 + Aac(6')-Ib-cr
Δ*ramR* *(qnrA1* + *aac(6')-Ib-cr)*	NS	S	S	S	deleted RamR + QnrA1+ Aac(6')-Ib-cr
***oqxR1* + *gyrA2* (pEX-K4)**	NS	NS	NS	NS	OqxR:Tyr109STOP + GyrA:Ser83Tyr Vector only
oqxR1* + *gyrA2* *(qnrA1)	NS	NS	NS	NS	OqxR:Tyr109STOP + GyrA:Ser83Tyr + QnrA1
oqxR1* + *gyrA2* *(aac(6')-Ib-cr)	NS	NS	NS	NS	OqxR:Tyr109STOP + GyrA:Ser83Tyr + Aac(6')-Ib-cr
Δ*ramR* + *gyrA1* (pEX-K4)	NS	NS	S	NS	deleted RamR + GyrA:Ser83Phe Vector only
Δ*ramR* + *gyrA1* *(qnrA1)*	NS	NS	NS	NS	deleted RamR + GyrA:Ser83Phe + QnrA1
Δ*ramR* + *gyrA1* *(aac(6')-Ib-cr)*	NS	S	NS	S	deleted RamR + GyrA:Ser83Phe + Aac(6')-Ib-cr
Δ*ramR* + *oqxR5* (pEX-K4)	S	S	S	S	deleted RamR + OqxR:Arg93Cys Vector only
Δ*ramR* + *oqxR5* *(qnrA1)*	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + QnrA1
Δ*ramR* + *oqxR5* *(aac(6')-Ib-cr)*	S	S	S	S	deleted RamR + OqxR:Arg93Cys + Aac(6')-Ib-cr

Quadruple acquisition events					
oqxR1 + gyrA2 (qnrA1 + aac(6')-Ib-cr)	NS	NS	NS	NS	OqxR:Tyr109STOP + GyrA:Ser83Tyr + QnrA1 + Aac(6')-Ib-cr
ΔramR + gyrA1 (qnrA1 + aac(6')-Ib-cr)	NS	NS	NS	NS	deleted RamR + GyrA:Ser83Phe + QnrA1 + Aac(6')-Ib-cr
ΔramR + oqxR5 + (qnrA1 + aac(6')-Ib-cr)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + QnrA1 + Aac(6')-Ib-cr
ΔramR + oqxR5 + gyrA1 (pEXK4)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + GyrA:Ser83Phe Vector only
ΔramR + oqxR5 + gyrA1 (qnrA1)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + GyrA:Ser83Phe + QnrA1
ΔramR + oqxR5 + gyrA1 (aac(6')-Ib-cr)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + GyrA:Ser83Phe + Aac(6')-Ib-cr
Quintuple acquisition event					
ΔramR + oqxR5 + gyrA1 (qnrA1 + aac(6')-Ib-cr)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + GyrA:Ser83Phe + QnrA1 + Aac(6')-Ib-cr

[a] *K. pneumoniae* Ecl8 is the parental strain in all cases.
[b] CIP, ciprofloxacin; LEV, levofloxacin; NOR, norfloxacin; OFL, ofloxacin
Zone diameter values for definition of susceptible (S) /non-susceptible (NS) designation were the means of three repetitions rounded to the nearest integer. Definitions were made by reference to susceptibility breakpoints set by the CLSI (29).

Table S5: Initial predictive rules and “reduced permeability correction” for ciprofloxacin susceptibility in *K. pneumoniae*

	Mutations identified in proteins			Presence of PMQR		Phenotype	“RP” Phenotype
Rule number	GyrA QRDR	OqxR	RamR	Qnr	Aac(6')-Ib-cr	(MIC [mg.mL^{-1}])	
1	+					S (0.25)	S (1)
2		+				S (0.25)	S (1)
3			+			S (0.125)	S (0.5)
4				+		S (0.063)	S (0.25)
5					+	S (0.016)	S (0.063)
6	+	+				NS	NS
7	+		+			NS	NS
8	+			+		S (1)	NS (4)
9	+				+	S (0.5)	NS (2)
10		+	+			S (0.25)	S (1)
11		+		+		NS	NS
12		+			+	S (0.5)	NS (2)
13			+	+		NS	NS
14			+		+	S (0.063)	S (0.25)
15				+	+	S (0.5)	NS (2)
16	+	+	+			NS	NS
17	+	+		+		NS	NS
18	+	+			+	NS	NS

19	+		+	+		NS	NS
20	+		+		+	NS	NS
21	+			+	+	NS	NS
22		+	+	+		NS	Ns
23		+	+		+	S (1)	NS (4)
24		+		+	+	NS	NS
25			+	+	+	NS	NS
26	+	+	+	+		NS	NS
27	+	+	+		+	NS	NS
28	+	+		+	+	NS	NS
29	+		+	+	+	NS	NS
30		+	+	+	+	NS	NS
31	+	+	+	+	+	NS	NS

+; mutation identified or PMQR present, blank; no mutation or PMQR absent, NS (Non-susceptible) and shaded blue. S; susceptible and not shaded as defined in Table S4

"RP" (Reduced Permeability) rules to be applied when the OmpF:OmpC ratio is <0.5.

MICs were determined when isolates were designated susceptible, RP correction is 4-fold, as described in the text. Susceptibility breakpoints applied for these isolates are MIC breakpoints defined by CLSI (29).

Table S6: Final predictive rules for ciprofloxacin susceptibility in *K. pneumoniae* clinical isolates.

	Mutations identified in proteins				Presence of PMQR		Phenotype (MIC [$mg.mL^{-1}$])
Rule number	1st GyrA QRDR	2^{nd} QRDR (ParC or GyrA)	OqxR	RamR	Qnr	Aac(6')-Ib-cr	
1RP	+						S (1)
2RP			+				S (1)
3RP				+			S (0.5)
4RP					+		S (0.25)
5RP						+	S (0.063)
6RP	+		+				NS
7RP	+			+			NS
8RP	+				+		NS (4)
9RP	+					+	NS (2)
10RP			+	+			S (1)
11RP			+		+		NS
12RP			+			+	NS (2)
13RP				+	+		NS
14RP				+		+	S (0.25)
15RP					+	+	NS (2)
16RP	+		+	+			NS
17RP	+		+		+		NS

18RP	+		+			+	NS
19RP	+			+	+		NS
20RP	+			+		+	NS
21RP	+				+	+	NS
22RP			+	+	+		Ns
23RP			+	+		+	NS (4)
24RP			+		+	+	NS
25RP				+	+	+	NS
26RP	+		+	+	+		NS
27RP	+		+	+		+	NS
28RP	+		+		+	+	NS
29RP	+			+	+	+	NS
30RP			+	+	+	+	NS
31RP	+		+	+	+	+	NS
32RP	+	+					NS (4)
33RP	+	+	+				NS
34RP	+	+		+			NS
35RP	+	+			+		NS
36RP	+	+				+	NS
37RP	+	+	+	+			NS
38RP	+	+	+		+		NS
39RP	+	+	+			+	NS
40RP	+	+		+	+		NS

41RP	+	+		+		+	NS
42RP	+	+			+	+	NS
43RP	+	+	+	+	+		NS
44RP	+	+	+	+		+	NS
45RP	+	+	+		+	+	NS
46RP	+	+		+	+	+	NS
47RP	+	+	+	+	+	+	NS

+; mutation identified or PMQR present, blank; no mutation or PMQR absent, NS (Non-susceptible) and shaded blue. S; susceptible and not shaded

Supplementary References

S1. Fu Y, Zhang W, Wang H, Zhao S, Chen Y, Meng F, Zhang Y, Xu H, Chen X, Zhang F. 2013. Specific patterns of gyr A mutations determine the resistance difference to ciprofloxacin and levofloxacin in *Klebsiella pneumoniae* and *Escherichia coli*. BMC Infect Dis 13:8.

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